Notes

Poster Sessions

There will be a poster exhibition in the vestibule throughout the meeting. Special Poster Sessions will be held on Wednesday, 3 March, 13.00 -14.30, and on Thursday, 4 March, 13.30 - 15.00. The authors are asked to be present with their poster at the respective time.
Posters are numbered according to the list given below. A-numbers in parentheses indicate the number of the respective abstract.

Poster Session 1 (Wednesday, 3 March, 13.00 - 14.30)

In vivo and in vitro effects of solid particles

1. **Transformation of the fetal rat alveolar type II cell line, FRLE, by a mutated K-*ras* gene, and studies on silica uptake and toxicity in FRLE cells (A 27)**
   U. Saffiotti, A.D. Knapton, M.E. Kaighn and A.O. Williams

2. **Effects of different silica samples in BALB/3T3/A31-1-1 cells: uptake, cytotoxicity and neoplastic transformation (A 28)**
   U. Saffiotti, N. Ahmed and A.O. Williams

3. **DNA damage in vitro induced by crystalline silica dust samples characterized for surface area and surface charge (A 29)**
   L.N. Daniel, Y. Mao and U. Saffiotti

4. **Immunohistochemical localization of TGF-ß1 in rat lung silicosis, adjacent alveolar type II hyperplasia and tumors (A 30)**
   A.O. Williams, K.C. Flanders and U. Saffiotti

5. **Response of the rat lung to inhaled versus instilled particles (A 31)**
   R.F. Henderson, K.E. Driscoll, R.C. Lindenschmidt, J.R. Harkema, E.B. Barr and I.Y. Chang

6. **Cyto- and genotoxic effects of insoluble particles in vitro (A 32)**
   M. Riebe-Imre, M. Aufderheide, S. Gärtner-Hübsch, A. Peraud and M. Straub,

7. **Chronic inhalation toxicity of talc in F344/N rats and B6C3F$_1$ mice (A 33)**
   C.H. Hobbs, K.M. Abdo, F.F. Hahn, N.A. Gillett, S.L. Eustis, R.K. Jones, J.M. Benson, E.B. Barr, M.P. Dieter, J.A. Pickrell and J.L. Mauderly

8. **Effect of arsenic exposure on alveolar macrophage function (A 34)**
   R.C. Lantz, G. Parliman, G.J. Chen and D.E. Carter

9. **Assessment of maximum exposure to soil contaminants during gardening (A 35)**
   I. Ollroge and Th. Reich

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10. **Effects of single and combined, subchronic exposure to ozone and soot particles on pulmonary surfactant phospholipids, associated protein A and on surface activity in rat lung lavage fluid (A 36)**
R. Klingebiel, U. Heinrich, I. Martin-Carrera, M. Dettmer, A. Kühn, W. Bartsch and H.G. Hoymann

11. **Morphometric evaluation of inhaled particles in lung sections (A 37)**
M. Kohler, M. Aufderheide and D. Ramm

12. **Goblet cell metaplasia in rat lungs after chronic exposure to particles (A 38)**
B. Kittel, D.L. Dungworth and U. Mohr

13. **Phagocytosis and chemotaxis of rat alveolar macrophages after a combined or separate exposure to ozone and carbon black (A 39)**
O. Creutzenberg, B. Bellmann, R. Klingebiehl, U. Heinrich and H. Muhle

14. **GM-CSF stimulated proliferation of rat alveolar macrophages and the effects of differing particulate burden levels in alveolar macrophages on the proliferative response (A 40)**
D. Pendergrass, Y.E. Valdez and B.E. Lehnert

15. **Influence of soot-associated organic compounds on pulmonary carcinogenicity of diesel exhaust in rats (A 41)**
K.J. Nikula, M.B. Snipes, W.C. Griffith, R.F. Henderson and J.L. Mauderly

16. **Are mixed coal mine dusts inert or toxic; new results to a classical unresolved experimental question (A 42)**
B. Rehn, J. Bruch, H.P. Song and Sh. Li

17. **Influence of the nature and the dose of dusts, on lung clearance. Experiences with a short time inhalation model (A 43)**
B. Rehn, H.P. Song, Ch. Li, W. Malkusch, B. Klein and J. Bruch

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Poster Session 2 (Thursday, 4 March, 13.30 - 15.00)

a) Fibre effects
b) Particles and tobacco smoke
c) Different approaches

18. **Toxic and chromosome damaging effects of natural and man-made mineral fibers in epithelial lung cells in vitro (A 55)**
A. Peraud and M. Riebe-Imre

19. **Cellular effects of mineral fibres (A 56)**
M. Aufderheide, M. Riebe-Imre, M. Straub and A. Peraud

20. **Increased incidence of DNA double-strand breaks and anti-DS DNA antibodies in blood of workers occupationally exposed to asbestos fibers (A 57)**
B. Marczynski, A.B. Czuppon, W. Marek, J. Ammon, G. Reichel and X. Baur

21. **Evidence of harmful health effects of occupational asbestos exposure (A 58)**
I. Trosic, L. Stilinovic and M. Saric

22. **Investigation on the biodurability of different man-made mineral fibres (A 59)**
B. Bellmann and H. Muhle

23. **Investigation on the biodurability of chemically different stone wool fibres (A 60)**
B. Bellmann, H. Muhle, O. Kamstrup and U.F. Draeger

24. **Quantitative recovery of fibers from the pleural cavity of the rat (A 61)**
E. Bermudez

25. **BRDU incorporation into rat lung cells following inhalation of man-made fibres and asbestos (A 62)**
K. Donaldson, D.M. Brown, R.C. Brown and A.R. Brody

26. **Pleural pathology in hamsters and rats exposed to instilled man-made vitreous fibers (MMVF) (A 63)**
F. Rutten, E. Bermudez, J. Mangum, B. Wong, O. Moss and J. Everitt

27. **Acute pleural response of hamsters and rats to inhaled ceramic fibers (A 64)**
J. Everitt, E. Bermudez, J. Mangum, B. Wong, F. Miller and O. Moss

28. **Characteristics of glass and amphibole fibres from the lungs of rats following inhalation exposure (A 65)**
R.C. Brown, J. Addison, B.G. Miller, K. Donaldson and J.M.G. Davis

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29. **Development and characterization of rat lung epithelial cell lines for toxicity studies (A 73)**
K.E. Driscoll, L.C. Deyo, T. Iype, J. Carter and D. Hassenbein

30. **Significance of length and diameter of a durable fibre on its carcinogenic potency (A 74)**
M. Roller and F. Pott

31. **Biokinetics of thorium and daughter radionuclides after deposition in the rat lung as fluoride and hydroxide (A 75)**
J.C. Moody, C.P. Davies and G.N. Stradling

32. **Modeling particle deposition in a bifurcating airway (A 76)**
B. Asgharian, S. Anjilvel and F.J. Miller

33. **A strategy for combining experimental, large-scale, and site-specific models to determine fiber deposition in animals and man (A 77)**
F.J. Miller, S. Anjilvel, B. Asgharian, J.S. Kimbell, T.R. Gelzleichter, B.A. Wong and O.R. Moss

34. **Bimodal bivariate lognormal distributions in the application of inhalation toxicology specific to the measurement of fiber and particle dosimetry (A 78)**
O. R. Moss, B. A. Wong and B. Asgharian

35. **Mathematical model of deposition, retention and clearance of insoluble particulates in the alveolar lung (A 79)**
C.L. Tran

36. **Histopathological findings in the rat respiratory tract in a 90-d inhalation study using sidestream smoke of the standard reference cigarette 2R1 (A 89)**
A. Teredesai and D. Prühs

37. **Influence of aging and surface contact on the composition of cigarette sidestream smoke - models for environmental tobacco smoke (A 90)**
P. Voncken, W. Stinn, H.-J. Haussmann and E. Anskeit

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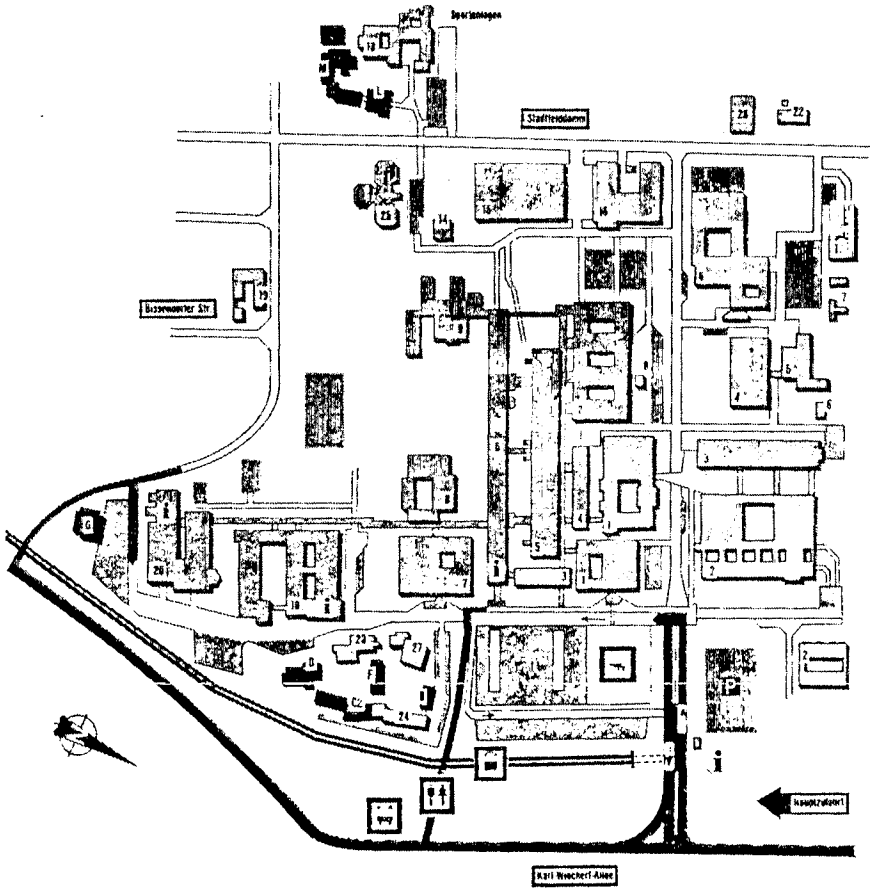

32

Key to Plan of Medical School

Clinical Area

K 1	Surgical Out-patients
K 2	Out-patients (West)
K 4	Laboratories
K 5	Examination, Treatment and Research Building (UBF-Bau)
K 6	Wards
K 7	Radiology
K 8	Physiotherapy
K 10	Paediatric Hospital
K 14	Immunologic Out-patients
K 20	Dental Hospital
K 25	KFH Building

Institutes and Teaching Area

I 1	Clinical Teaching Block
I 2	Preclinical Teaching Block
I 3	Theoretical Institutes I
I 4	Research Technology
I 5	Central Animal Laboratory
I 6	Theoretical Institutes II
I 7	Kennels
K 18	School for Medical Assistants
K 19	School for Medical Assistants (Bissendorfer Str.)

Residential Area

Residential	Block B
	Block C2
	Block D
	Block F
	Tower Block G
	Tower Block L
	Block M

Administration

Z 2	Day Nursery
K 3	Hospital Administration (Patient Registration)
I 8	Bottled Gas Store
I 9	Ether Store
K 15	Cafeteria (Mensa)
K 16	Technical Administration
K 17	Laundry
K 22	Boiler Room
K 23	Multipurpose Building (House E)
K 24	Multipurpose Building (House C1)
K 27	Administration Building (House A)
K 28	Refuse Disposal

33

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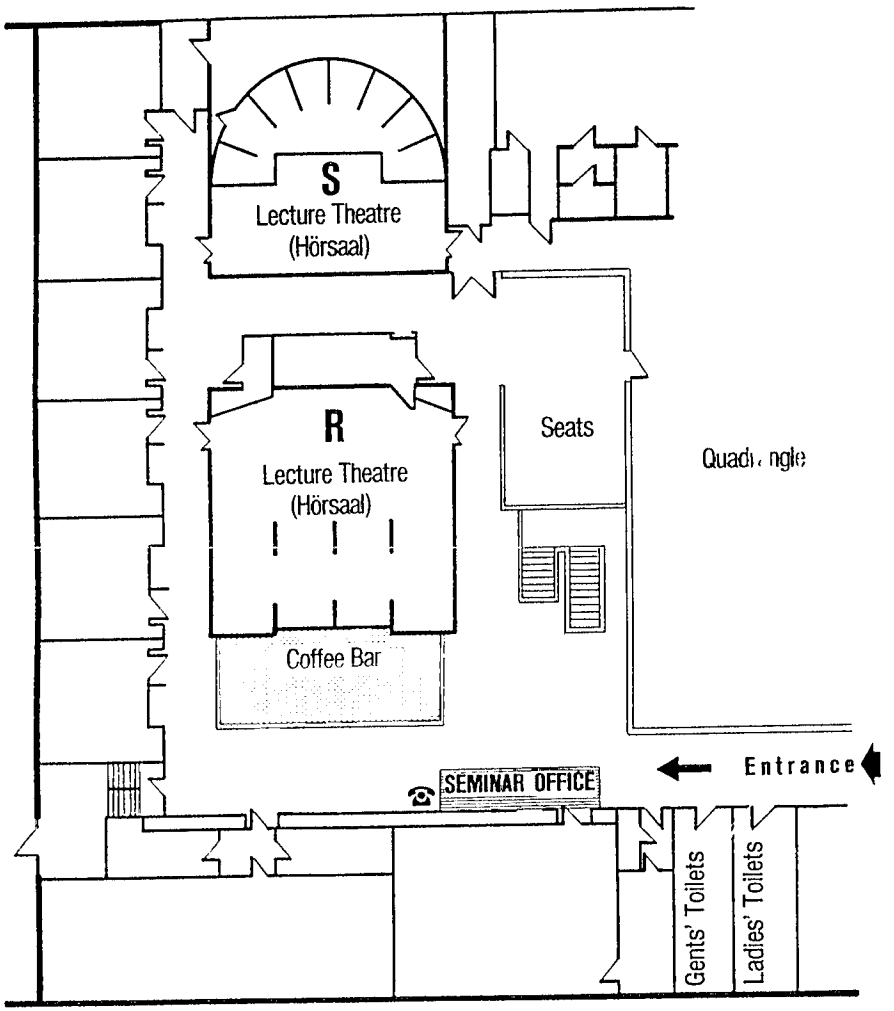
Room Plan



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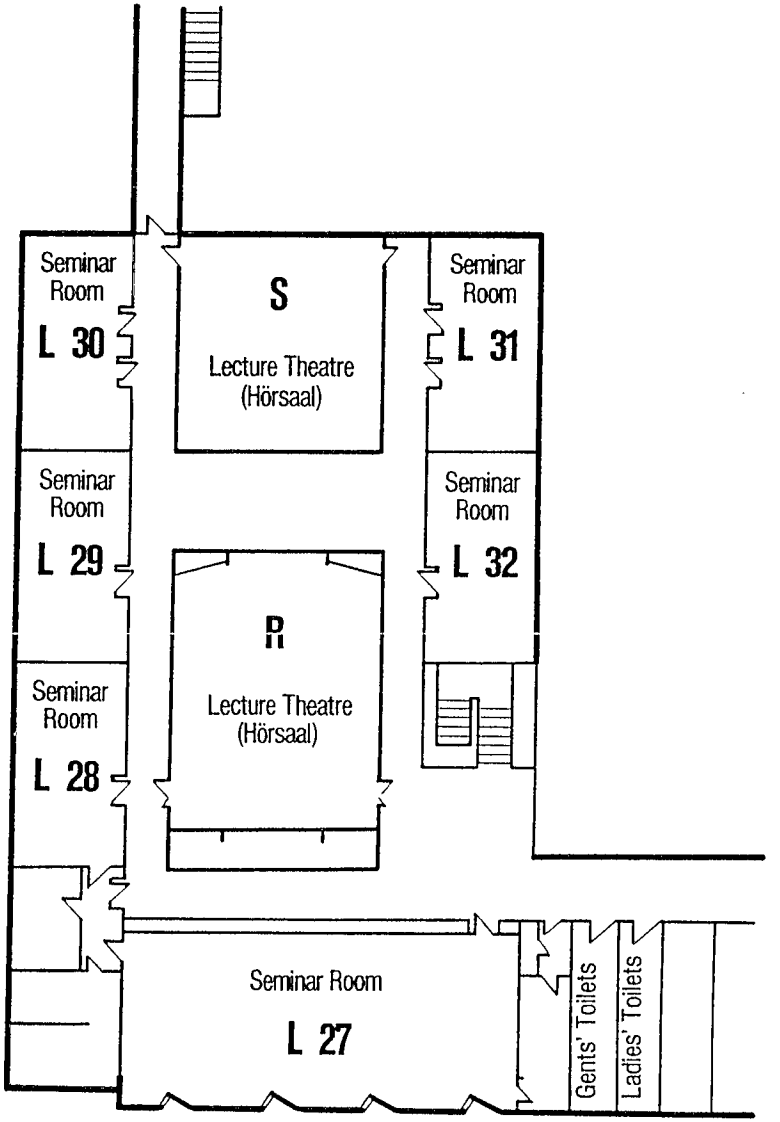
Main Floor



First Floor

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A 1

Particle and Fibre-Induced Lesions - an Overview -

Agnes B. Kane, Brown University, Providence, RI 02912, USA

Inhalation of particulate and fibrous minerals causes a spectrum of pulmonary diseases. Crystalline quartz or silica and asbestos fibers will be used as prototypes to explore the mechanisms responsible for these diseases. Silica exposure is associated with acute lipoproteinosis, nodular pulmonary fibrosis, increased susceptibility to infections caused by mycobacteria and fungi, and possibly, an increased incidence of lung cancer. Asbestos fibers produce interstitial pulmonary fibrosis, diffuse fibrosis or fibrous plaques of the visceral and parietal pleura, an increased incidence of lung cancer, and malignant mesothelioma. The initial target cells to interact with particulate or fibrous minerals in the lungs are macrophages. These cells are actively phagocytic and release several potential biochemical mediators of lung disease: reactive oxygen species, arachidonic acid metabolites, chemotactic factors and other cytokines, lysosomal enzymes, neutral proteases, and growth factors. These biochemical mediators are important in normal lung defenses and they aid in healing acute lung injury. In response to the chronic persistence of mineral particles and fibers, there is prolonged release of these biochemical mediators resulting in repeated episodes of lung injury leading to nodular or diffuse fibrosis. Concommitant exposure to asbestos fibers and cigarette smoke reduces clearance of particulates and fibers from the lungs and may facilitate activation of chemical carcinogens. Fibers may also interfere with the normal regulatory pathways for cell growth and differentiation leading to carcinomas arising in the bronchial epithelial lining. Malignant mesotheliomas occur less frequently after exposure to asbestos fibers and are not synergistic with cigarette smoking. Mesothelial cells are damaged by reactive oxygen species released from macrophages during phagocytosis of asbestos fibers. Mineral fibers may also directly or indirectly damage the mitotic apparatus in mesothelial cells that are proliferating in response to injury. This genetic damage may activate cellular protooncogenes or inactivate tumor suppressor genes. Further exploration of these biochemical and molecular changes triggered by inhalation of particulate and fibrous minerals will reveal the sequence of events leading to development of fibrosis, lung cancer, and malignant mesothelioma.

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A 2

Mechanisms and Significance of "Particle Overload"

Paul E. Morrow, University of Rochester Medical Center, Rochester, NY 14642, USA

Following a brief historical review of key studies with inhalation exposures resulting in high pulmonary dust burdens in Fischer 344 rats and producing the condition of dust overload, several investigations providing possible mechanistic information on dust overload will be discussed including recent findings from a number of inhalation studies performed at the Fraunhofer Institute in Hannover. A hypothesis describing lung overload in terms of the average particle volume per alveolar macrophage will also be briefly described. The presentation will conclude with a description of the present implications, both real and imagined, of dust overload on the toxicological and regulatory communities.

A 3

Toxicokinetics of Solid Particles in Chronic Studies: An Overview

H. Muhle, Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, FRG

This paper gives an overview on the retention and clearance of solid particles in various studies which were run in different laboratories. The particles used include: diesel soot, carbon black, toner for copy machines, titanium dioxide, and quartz. Animal species used include rats, mice and Syrian golden hamsters. The presented data serve as a data base for studies presented in this meeting describing non-neoplastic and neoplastic changes.

It is analyzed under which conditions the phenomenon of "particle overload" of lungs was observed. This effect is caused by an immobility and dysfunction of macrophages resulting from an excessive uptake of particles. The reduced immobility was measured by the elimination kinetics of inhaled radioactive tracer material. The relationship between retained dust in the lung and the retardation of the macrophage-mediated clearance gives information on the intrinsic toxicity of the inhaled particles, e.g. for quartz.

The reversibility of the overloading effect was investigated in a subchronic exposure study to toner at 10 and 40 mg/m^3 followed by a 15-month post-treatment observation period. Results did not show a recovery of the "particle overload" effect of the lung.

Particle overload of lungs is an important issue in the interpretation of inhalation toxicological studies. It is an open question whether data from studies under overloading conditions can be useful for the prediction of health effects in man.

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A 4

Non-Cancer Effects of Chronic Inhalation Exposure of Animals to Solid Particles

J. L. Mauderly, R. F. Henderson, K. J. Nikula and M. B. Snipes, Inhalation Toxicology Research Institute, P.O. Box 5890, Albuquerque, NM 87185, USA

Information on non-cancer effects of exposure of animals to solid particles comes primarily from rodents. Chronic exposure of rodents to respirable, poorly-soluble, non-fiber, non-quartz particles causes few abnormalities if the deposition rate does not exceed macrophage clearance capacity. If macrophage clearance is exceeded, particles accumulate progressively, primarily in macrophages, which tend to form centrilobular aggregates in rats, but remain more disperse in mice. Progressive particle accumulation is accompanied by dose-related, multifocal inflammation, cytotoxicity, epithelial hyperplasia, squamous metaplasia, and fibrosis. These changes are reflected by increased concentrations of cells, cytoplasmic and lysosomal enzymes, mediators of inflammation, and protein in lavage fluid. In rats, focal lseions are often intersperded with relatively normal alveoli. Substantial focal histopathology can occur before a restrictive respiratory function impairment is measurable. Pulmonary lymph nodes may become enlarged with particles, but antibody formation may not be affected. Available data suggest that developing lungs are not more susceptible to particle exposures than adult lungs, and that lungs with pre-existing emphysema are not more susceptible to particle exposures than normal lungs. An increasing quartz content renders particles more cytotoxic, causing greater histopathology and leading too granuloma formation. (Supported by the U.S. Department of Energy and the Health Effects Institute.)

A 5

Carcinogenic Effects of Solid Particles

U. Heinrich, Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, FRG

The co-carcinogenic effect of intratracheally (i.tr.) administered nuisance particulates like titanium dioxide (TiO_2), carbon (C), ferric oxide (Fe_2O_3) or aluminum oxide was already demonstrated in hamsters almost 20 years ago (Henry et al. 1974, Stenbäck et al. 1976). Particle-related lung tumors in rats were reported for the first time after inhalation exposure to 200 mg/m^3 coal dust (Martin et al. 1977). A similar tumor response occurred after inhalation of 250 mg/m^3 TiO_2 (Lee et al. 1985) and after i.tr. instillation of 10 mg of activated carbon (Kawabata et al. 1986). Since the unexpected high incidence of lung tumors found in rats exposed to diesel engine exhaust could not be explained solely by the low content of carcinogenic polycyclic aromatic hydrocarbons (PAH) of diesel soot, the hypothesis was generated that the carbonaceous core of diesel soot might be responsible for the diesel exhaust-related tumor response (Pott and Heinrich, 1987). This hypothesis proved to be right when rats exposed to 6 mg/m^3 carbon black particles (CB), almost completely devoid of organic material for 10 months, also developed lung tumors (Heinrich et al. 1991). Comparing CB, TiO_2 and diesel soot all consisting of agglomerates of primary particles less than 50 nm in size, similar lung tumor rates were observed in rats exposed to 7 - 10 mg/m^3 particles; no increased lung tumor rate was seen in dust-exposed mice (Heinrich et al. 1992). Comparing the various inhalation studies on the carcinogenic effect of non-fibrous particles in the rat lung, it becomes obvious that dust particles composed of very fine primary particles (< 50 nm) cause tumors at a clearly lower particle exposure concentration and particle lung load than dusts which consist of larger-sized but still respirable compact particles. An exception to this rule is quartz dust which, because of its high inherent cytotoxicity, acts more like a conglomerate of small primary particles (Muhle et al. 1989).

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A 6

Pathologic Effects of Inhaled Particles in Rat Lungs: Associations Between Inflammatory and Neoplastic Processes

D.L. Dungworth[1], U. Mohr[2], U. Heinrich[3], H. Ernst[3] and B. Kittel[3], [1]University of California, Davis, CA, USA, [2]Institute of Experimental Pathology, Hannover Medical School, Hannover, FRG and [3]Fraunhofer Institute of Toxicology and Aerosol Research, Hannover, FRG

The associations between inflammatory, hyperplastic and neoplastic lesions in the lungs of particle-exposed rats were studied by special review of a representative sample of exposure regimens. These were female Wistar rats which had been exposed 17-18 hrs/day for 5 days/week from the age of 10 weeks to the following regimens: 1) 6 mg/m^3 carbon black particles for 10 or 20 months (n = 72); 2) 7.5 mg/m^3 diesel exhaust particles for 24 months (n = 100); 3) average of 10 mg/m^3 titanium dioxide for 24 months (n = 100). Following exposure, all rats were allowed to live in clean air up to a total of 30 months; 361 rats in clean air served as controls. These particle effects were compared with rats which were exposed for 10 or 20 months (n = 72) to coal tar/pitch (T/P) condensation aerosol free of insoluble particles and which contained 50 µg benzo[a]pyrene (BaP)/m^3 among other polycyclic aromatic hydrocarbons.

All particle-exposed lungs had moderate to severe inflammation which was characterized by multifocal bronchioloalveolar hyperplasia, alveolar histiocytosis and focal or segmental fibrosis. Small foci of squamous metaplasia occurred rarely. Prevalence of bronchioloalveolar tumours (adenoma and carcinoma) ranged from 3 to 17 % in the various exposure regimens and for squamous tumours (benign cystic tumours and squamous cell carcinomas) the range was 7 to 22 %.

In contrast, rats exposed to the tar/pitch aerosol containing BaP frequently had multifocal and often massive proliferation of squamous cells which ranged (often in the same lung) from squamous metaplasia through the benign cystic tumours to frankly malignant squamous cell carcinoma. Although the prevalence of squamous cell tumours was 96 % in the group exposed to T/P aerosol for 20 months, bronchioloalveolar tumours were rare (3 %). The fact that the range of squamous proliferation in particle-exposed rats fitted within the continuum of lesions observed in the BaP-exposed rats helped justify the interpretation of the large cystic lesions as benign tumours (ie neoplasia rather than simple hyperplasia).

The implications of these findings for particle-induced carcinogenesis in the rat lung and for human risk assessment will be discussed.

A 7

Animal Inhalation Studies with Fibres

J. M. G. Davis, Institute of Occupational Medicine, Edinburgh, Scotland

Within a few years of the general recognition that the industrial use of asbestos could result in severe pulmonary fibrosis (asbestosis) animal studies using inhalation techniques were commenced. These early studies confirmed that it was possible to reproduce the lesions of asbestosis in experimental animals and produced evidence that long asbestos fibres were much more pathogenic than short. Owing to the limitations of animal husbandry, the early studies failed to demonstrate that asbestos fibres could be carcinogenic as well as fibrogenic. By the time it was realised that asbestos could cause both pulmonary carcinomas and mesotheliomas in humans, laboratory studies were more sophisticated. Several workers were able to demonstrate that rats would develop both these conditions following asbestos inhalation. Disease development was dose-related but in rats there is little difference in the pathogenicity of the different asbestos types when dose is estimated by mass. The importance of long fibres in disease development was amplified by injection studies and finally confirmed by up-to-date inhalation experiments. It appears that the most pathogenic fibres are > 20 µm with fibres < 5 µm in length having little harmful potential. Recent studies have concentrated on man-made mineral fibres and results have appeared controversial when compared to injection experiments. After injection, all MMMF samples containing long thin fibres appear carcinogenic in rats but when administered by inhalation most appear innocuous. This difference appears due to the varying durability of fibres in lung tissue. Following inhalation only those that can survive in the lung long enough to accumulate can cause disease regardless of their dimensions. Injection studies, using large doses and bypassing all clearance mechanisms can produce tumours with fibres of relatively low durability. Nowadays, it is realised that both the dimensions of airborne fibres and their durability need to be taken into account when predicting possible hazards to humans.

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Carcinogenicity Studies: Relevance of Unphysiologic Routes

F. Pott and M. Roller, Medizinisches Institut für Umwelthygiene an der Heinrich-Heine-Universität Düsseldorf, Düsseldorf, FRG

Intratracheal instillation or injection of substances are called "unphysiologic" exposure routes. There is a controversial debate whether results achieved by these methods give evidence of hazardous effects that should be extrapolated qualitatively to humans. Regarding the carcinogenicity of fibers thinner than 1 µm, it is well-known that the sensitivity of long-term inhalation studies in rats is very low compared with man; moreover, thicker fibers cannot be tested by inhalation because they cannot pass the effective nose filter of rats. Although there are several open questions on the mechanisms in fiber carcinogenesis and the cancer risk from different fiber types, all the available results with asbestos fibers, non-asbestos fibers and non-fibrous particles lead to the conclusion that there is a qualitatively analogous tumor-producing reaction of the lung and the serosal tissue of rats to elongated particles. The abdominal cavity has a much higher capacity for testing materials than the pleural space of one side of the thorax and it is possible to find dose-response relationships. It would be of great interest to learn whether the ranking order of the carcinogenicity of different fiber types is similar in the lung and in the peritoneal cavity.

High doses of non-fibrous dusts (e.g. diesel soot, carbon black, iron oxides) were not found to be carcinogenic after intraperitoneal injection but induced lung tumors after intratracheal instillation and partly also after inhalation in rats.

Fate and Translocation of Fibres

Kent E. Pinkerton and James D. Crapo, University of California, School of Veterinary Medicine, Davis, CA 95616 and Duke University Medical Center, Durham, NC, USA

The fate of mineral fibres in the respiratory system is highly dependent on the physical properties of the inhaled material. Characteristics of size, aerodynamic behaviour, and durability dictate the eventual outcome of fibre-cell interactions in the lungs. The first line of defence against inhaled fibres is the fluid lining of airways and alveoli which facilitate entrapment and removal. Macrophages in these same locations also serve to engulf and/or impair movement of fibres from the air spaces to the alveolar walls and septa. However, when these defence mechanisms fail, as is inevitable with fibres which penetrate deeply into the lungs and are of a size too large for a single cell to ingest, long-term consequences are the translocation of fibres through the epithelium into the interstitium with eventual entombment in a matrix of collagenous material. Many fibres remain in close proximity to original sites of deposition in the lungs. A distinct pattern of tissue remodeling within unique regions of the lungs and along alveolar ducts is also reflective of the number, size and mass of fibres present in each microdomain of the lung parenchyma. Interstitial clearance of fibres is highly dependent on fibre size and is exclusively a function of phagocytic and immune effector cells present in the interstitium. Eventual removal of long-term resident fibres is likely to occur primarily through the gradual dissolution of the fibre.

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A 10

Factors Controlling the Biological Potential of Inorganic Dusts; Surface Chemistry and Character

A. M. Langer and R. P. Nolan, Environmental Sciences Laboratory - Applied Sciences Institute, Brooklyn College - City University of New York, Brooklyn, New York 11210, USA

The properties of quartz and rutile dusts which control their **in vitro** behavior have been experimentally determined. Material source, and properties superimposed by industrial processes impart surface character and biological potential.

Source of the mineral, particle size distribution of crushed or synthetically grown powders, resultant surface crystallinity and their chemical functionalities, chemical and thermal histories, control their behavior. Biological activity of an inorganic powder is controlled by its physicochemical state.

Quartz and rutile exhibit membrane activities directly attributable to their separate and non-interfering protonated and ionized sites on their respective surfaces. These in turn control the minerals' binding properties which reflect membrane communication potential. Both quartz and rutile dusts exhibit e.g., proton donating properties. The activity of the titania surface was successfully blocked through binding of proton-accepting polymers, e.g., 2-polyvinylpyridine-N-oxide. The same effect has been observed for quartz powders.

The ionized site on the surface of quartz and titania also participates in membrane recognition.

2057358115

A 11

Pulmonary Macrophages: Phenomena Associated with the Particle "Overload" Condition

B. E. Lehnert[1], R. J. Sebring[1] and G. Oberdörster[2], [1]Los Alamos National Laboratory, Los Alamos, NM 87545 and [2]University of Rochester, Rochester, NY 14642, USA

Numerous lines of evidence support the generalization that alveolar macrophage (AM)-mediated particle clearance, or the transport of particle-containing AM from the alveoli out of the lung via the mucociliary apparatus, is a prominent mechanism that determines the pulmonary retention characteristics of relatively insoluble particles. As well, several studies have shown that the alveolar deposition of excessive burdens of particles with even low intrinsic cytotoxicity can result in impairments of the AM-mediated particle clearance mechanism and the development of pathologic disorders including pulmonary fibrosis and lung cancer. In this report, we review evidence consistent with the idea that the volume of particles contained in AM is associated with the ability of these cells to perform their lung clearance function. Other mechanisms, however, may also contribute, at least in part, to retardations in the clearance of particles from the lung during particle overload. Using a condition of particle overload brought about by a subchronic exposure of rats to ultra-fine titanium dioxide as an experimental model, ultrastructural and other evidence have been obtained that shows an association between particle overload and: 1) the occurrence of particles in extra-alveolar macrophagic compartments, 2) the occurrence of aggregates of particle-containing AM in alveoli, 3) Type II cell hyperplasia in alveoli that contain the AM aggregates, and 4) an occlusion by Type II cells of the pores of Kohn, which conceivably may effectively eliminate a pathway through which AM may gain access to neighboring alveoli and perhaps travel on their way to the mucociliary apparatus. The last component of the report concerns potential mechanisms by which elevations in the sizes of the AM and interstitial macrophage populations that are observed in conditions of particle overload and pulmonary fibrosis may be sustained, with particular emphasis being placed on the proliferative abilities of the AM and how such proliferation may be regulated by lung fibroblast-derived and other cytokines. [This work was supported by the U.S. DOE and the NIH.]

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A 12

Contribution of Macrophage-Derived Cytokines and Cytokine Networks to Particle-Induced Inflammatory Cell Recruitment

K. E. Driscoll, J. K. Maurer, D. Hassenbier and J. Poynter, Human & Environmental Safety Division, The Procter & Gamble Company, Cincinnati, OH 45239, USA

Alveolar macrophage-derived lipids and proteins play an important role in maintaining the normal homeostasis of the lung and can contribute to pulmonary injury and disease. Tumor necrosis factor α (TNF) is a macrophage-derived cytokine with immunomodulatory and inflammatory activity. Studies with pneumotoxic agents in animals (e.g., silica, asbestos, bleomycin) and in humans (e.g., asbestos, silica) indicate TNF may play an important role in the pathogenesis of interstitial lung disease. Our laboratory has examined the relationship between particle exposure in the rat and release of cytokines including TNF by lung macrophages. We have demonstrated increased release of TNF by lung macrophages after in vivo exposure to toxic particles such as silica or high lung burdens of materials such as titanium dioxide or aluminum oxide. Particle-induced macrophage TNF release was positively correlated with neutrophilic inflammation. Our subsequent studies demonstrated passive immunization of rats with anti-TNF antibody attenuated silica and titanium dioxide elicited neutrophil responses. These findings indicate TNF plays an important role in particle-induced neutrophil recruitment. One mechanism by which TNF may act to recruit inflammatory cells is by activating release of chemotactic factors for these cells. We recently demonstrated TNF can stimulate rat alveolar macrophages, rat lung fibroblasts or a rat lung epithelial cell line to secrete MIP-1α, MIP-2 and MCP-1, peptides which are highly chemotactic for neutrophils and monocytes. Acute and subchronic exposure of rats to silica or titanium dioxide under conditions which increase TNF were also shown to increase expression of these chemotactic cytokines in lung. Thus, alveolar macrophage-derived TNF plays an important role in lung defense and disease through recruitment of inflammatory cells. The TNF-mediated inflammatory response likely involves both autocrine and paracrine communication pathways between macrophages, lung epithelial cells and fibroblasts.

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A 13

Mechanisms of Asbestos-Mediated DNA Replication and Cell Proliferation

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Occupational exposure to asbestos is associated with the development of mesothelioma, lung cancers, and pulmonary fibrosis (asbestosis). All of these diseases are characterized by abnormal cell proliferation of various target cells. Recently, we have been exploring the molecular mechanisms of asbestos-induced cell replication in cultures of rodent mesothelial and tracheal epithelial cells. Both crocidolite and chrysotile asbestos appear to cause increased proliferation in these cell types by at least two pathways. The first involves an increase in steady-state mRNA levels and enzyme activity of ornithine decarboxylase (ODC), a rate limiting enzyme resulting in subsequent accumulation of polyamines, growth regulatory molecules which must increase in cells for cell division to occur. Since addition of antioxidants ameliorates these changes, biosynthesis of polyamines by asbestos appears mediated by a pathway involving active oxygen species (AOS). A second mechanism of asbestos-induced proliferation in mesothelial cells involves the increased and persistent induction of c-*fos* and c-*jun* protooncogenes, a hallmark of the early response gene pathway. Increases are more striking after exposure to crocidolite in comparison to chrysotile asbestos and are not observed after exposure to particles or synthetic materials such as refractory ceramic or man-made vitreous fibers. Asbestos causes increased mRNA accumulation of c-*jun*, but not c-*fos*, in tracheal epithelial cells. In both cell types, induction of protooncogenes is accompanied by coincidental increases in protein factors that bind specifically to the DNA sites that mediate gene expression by the AP-1 family of transcription factors. We are currently pursuing the question of whether or not AOS are involved in the chronic activation of early response genes.

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Cell-Cell Interactions and the Role of Epithelial Cells in the Pulmonary Response to Particulate Injury

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The type II cell plays an important role in the response of the alveolar epithelium after lung injury through its synthesis and secretion of pulmonary surfactant, and by acting as the stem cell for the replacement of damaged type I epithelial cells. Recent evidence has suggested that epithelial cells may participate in aspects of the inflammatory response and in the regulation of fibroblast growth during pulmonary fibrosis through the production of and response to specific mediators or "growth factors". To address these issues a number of lung injury models have been utilized and the proliferation and growth factor production by type II cell *in vivo* and *in vitro* have been measured. Adult rabbits, mice or rats were exposed to 100 % O_2 doses of radiation from 2.5 to 17.5 Gy to both lungs or were exposed to presumptive fibrogenic and nonfibrogenic particles and fibers by inhalation or instillation. At varying times after treatment, type II cells were isolated by proteolytic digestion and placed in culture or deposited on slides. Experiments were also performed by exposure of isolated type II cells to injurious stimuli *in vitro*. Growth factor and cytokine production was measured by immunoblot analysis of conditioned media from cultured cells or directly on cells placed on slides. *In situ* hybridization was used to assess cell specific expression of growth factor mRNA. In addition, CM from type II cells was evaluated for its ability to alter the proliferation of normal lung fibroblasts and untreated type II cells. Type II cells isolated from animals exposed to hyperoxia, ionizing radiation, and fibrogenic particulates were found to produce increased amounts of TGFα, TGFβ, PDGF, IL-1 and fibronectin. TGFα and TGFβ production was increased 2 - 3 fold compared to control cells after O_2 and up to 4 weeks after radiation exposure. CM from injured type II cells enhanced fibroblast proliferation and inhibited type II cell growth, while control CM was inhibitory to both cell types. These results suggest that type II cell growth factor production may be significant in the pathogenesis of pulmonary fibrosis. (Supported in part by HL 36543, ES 04872, CA 27791, CA 11051, ES 01247 and NASA-NSCORT grant NAGW-2356.)

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In Vitro Approaches to Screening Particles for Toxicity

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This contribution primarily intends to review the methods developed so far for studying and detecting toxic effects of environmental particulate matters using in vitro cultivated cells.

Recently one has come to the conclusion that - as long as laboratory animals cannot mimic humans sufficiently - the procedure for data extrapolation from animal experiments to the human situation should be assisted for accuracy to a greater extent by knowledge of the mechanisms involved in toxicity. In this respect in vitro methods should be able to answer the open questions. A more guaranteed access to human cells/tissues than ever may also promote the improvement and applicability of these methods.

In applying in vitro methods for screening particulates for toxicity several basic questions may arise concerning the cell systems and the techniques of cultivation: 1. Do the target cells need to be functionally differentiated, i.e., is it necessary for the in vitro target cells to mimic the real in vivo target cells? 2. Should the cell system consist of a single cell population or of a cocultivation of defined multiple cell populations? 3. Should the cell populations to be used be derived from the respiratory organs? 4. Do the cell systems need (a) specific matrices(x) or specific additives in the medium? 5. Should the screening be carried out under combined exposure to any gaseous compounds (e.g. tobacco smoke)? - In the actual test procedures these questions occur mostly interrelated, and which answer is selected for setting up test procedures may in principle be a matter of choice depending on the chemical and physical nature of particles and the type of toxicity. Nevertheless, there appear to be certain desirable preconditions or rules to be met. For instance, on points 1 and 4: The level of manganese superoxide dismutase (MnSOD) is reportedly high in the type II pneumocytes in vivo. When the effects of silica dusts are looked at using in vitro cultivated type II pneumocytes which have a low level and inducibility of MnSOD, we will obtain a much different picture of silica toxicity as compared with that in vivo.

In conclusion, the data presently obtained in in vitro toxicity studies require very careful interpretation by taking into account the cell type, species, state of functional potency, presence or absence of other cocultured cell types, and utilization of specific matrix and/or differentiation-stimulating factors. To make data comparison more feasible, standardization and validation of various test procedures will soon be necessary.

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Effects of Fullerenes on Alveolar Macrophages in Vitro

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Fullerenes are a newly discovered modification of pure carbon consisting of cage like molecules composed of well defined numbers of carbon atoms. Fullerenes with 60 atoms (C_{60}) are the smallest molecules of this type, looking like a football - a polygon with 60 vertices and 32 faces, 12 of which are pentagonal and 20 hexagonal. These molecules can form crystals of various shapes, also needle like structures could be detected with the material used in our laboratory. Fullerenes are readily soluble and vaporizable molecules remaining stable in air and are perfectly suited to a wide range of potential (technical) applications, e.g. in electronics or even pharmacy. In order to obtain some first and preliminary knowledge on possible toxic effects in the lung after inhalation in vitro studies with cell cultures were begun. Special emphasis has been paid to the fullerene-induced release of cytokines and chemotaxins as indicators for a pro-inflammatory effect of the particles. The material, C_{60} and C_{60-70}, was prepared in an arc between two graphite electrodes in a helium atmosphere and subsequent chromatographic separation. For the biological studies the material (100 µg/ml) was incubated after sonication for 4 hours with HL-60 macrophages (HL-60-M), a human cell line differentiated to macrophages by Calcitriol, which has been shown to be a useful test system in our institute. Bovine alveolar macrophages (BAM) have also been used. The release of Tumor Necrosis Factor (TNF), Interleukin-6 (IL-6) and Interleukin-8 (IL-8) was assayed by commercially available ELISA kits (Biermann, Bad Nauheim) or by bioassay with WEHI cells in the case of BAM. The effects of fullerenes were compared with those of the standard stimulus Lipopolysaccharide (LPS) known as inductor of strongly positive reactions. Even after 20 hours of incubation the C_{60} fullerenes had no influence on the viability of HL-60-M. However, TNF, Il-6 and IL-8 could be detected in considerable concentrations in the culture supernatants already after 4 hours. The extent of the reaction of the cells to C_{60} and C_{60-70} corresponded to that after stimulation with with LPS. In further experiments we could show that the effects of C_{60} corresponded also to those of quartz particles used as positive control. Qualitatively, there were no great differences between the reactions of the cells towards C_{60}- and C_{60-70}-fullerenes. A very interesting result was obtained when the fullerene particles had been sonicated first together with the natural surfactant component Dipalmitoyl-phosphatidylcholine (DPL). In this case no effects could be observed at all. From these results it can be concluded that at least the fullerene preparations used in our experiments are toxic to alveolar macrophages. The fact that pretreatment of the particles with DPL completely abrogates their toxicity in vitro suggests that the effects could be related to some properties of the particle surface. In the future, the influence of contaminants and modifications of the materials on their biological effects has to be investigated.

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In Vitro Effects of High T_c Superconducting Materials and their Components on some Immunofunctions of Macrophages

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During the past few years we have obtained evidence that particles of high T_c superconducting materials (HTS, mainly $YBa_2Cu_3O_{6-7}$) can cause functional and structural disorders in alveolar macrophages, including disturbance of certain immunofunctions. The particles as such are rather insoluble in water but dissolve rapidly inside phagolysosomes. The present study mainly deals with the in vitro effects of those HTS which contain various rare earth elements, barium and copper on macrophages. Two HTS types without rare earth components were also used. The influence of the respective ions is compared with that of the integer particles in order to elucidate the contribution of each single element to the total effect.

The cells were bovine alveolar macrophages (BAM) or HL-60 cells differentiated to macrophage like cells by Calcitriol (HL-60-M). The following materials were tested: $YBa_2Cu_3O_{6-7}$; $YBaCuO_{3.5}$; $LaBaCuO_{3.5}$; $PrBa_2Cu_3O_X$; $ErBa_2Cu_3O_X$; $Tl_2Ba_2Ca_2Cu_3O_X$; $Bi_{1.8}P_{0.2}Ca_2Sr_2Cu_3O_X$. The chlorides of the respective constituents were used in concentrations up to 100 µM. The concentration of HTS was up to 100 µg/ml, the duration of incubation up to 24 hours. The production of the mediators IL-8 and TNF was assayed by ELISA-Kits. Chemotactic response and the release of a chemotactic factor (ChF) by BAM (which is not identical with IL-8) were tested by a microchemotaxis chamber or by the leading front method.

$LaBaCuO_{3.5}$ inhibits the chemotactic migration of BAM towards Zymosan activated serum. This can fully be explained by the La component, which is a potent inhibitor of chemotactic migration. ChF plays an important role for the interaction of macrophages with each other and, e.g., with neutrophil granulocytes. When BAM are incubated for 20 h with $LaBaCuO_{3.5}$, they release this factor into the culture medium. Also in this respect, we could show that La is responsible for the effect. IL-8 is produced by HL-60-M after incubation with all types of HTS. In this case no systematic IL-8 releasing effect of the single ions was found. A similar situation exists for the release of TNF by HL-60-M: a marked TNF production is measurable with all HTS materials, but only the Tl ion is effective by itself.

The results show that HTS can disturb important immunofunctions of macrophages. The release of chemotactic factors and TNF is a strong indicator of a pro-inflammatory action of the particles. The rare earth components alone can be responsible for the effects on chemotactic mobility and release of ChF, perhaps by interference with the Ca metabolism. Most probably the induction of IL-8 and TNF is due to a combined influence of two or more of the constituents of HTS.

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